A putative hybrid of the Murray Crayfish, *Euastacus armatus* (Crustacea: Decapoda: Parastacidae)

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ABSTRACT

An unusual population of freshwater crayfish of the genus *Euastacus* found in the East Buffalo River, Victoria, is morphologically distinguishable from other populations, and in particular has a marbled or camouflage pattern on the carapace. The cheliped dactylar spine counts and the number of zygocardiac teeth anterior to the ventral ear of the zygocardiac ossicle (TAP) are somewhat reduced but these values overlap with the range of the most similar species (*E. armatus*). Genetic sequences from the CO1 and 16S mitochondrial DNA regions were also undertaken, and the results indicated that these animals may represent an aberrant population of *E. armatus* (the Murray Crayfish) or perhaps a hybrid between *E. armatus* and *E. woiwuru*, two species that are found downstream and upstream, respectively, of the population investigated. The conservation status of the population remains unclear. ❐ *Decapoda, Parastacidae, mitochondrial DNA, Buffalo River, conservation, Murray Cray, hybrid.*

Australian freshwater crayfish are an ancient and diverse group that are increasingly in need of conservation (Horwitz 1990a; Merrick 1997; O'Brien 2007). Spiny freshwater crayfishes of the genus *Euastacus* are found on the east coast of Australia, from the Great Dividing Range in Victoria to isolated mountains in northern Queensland (Clark 1941; Morgan 1986, 1988, 1997). New species have recently been described from north-eastern New South Wales (Coughran 2002, 2005). Most species in the genus are considered short-range endemics (Harvey 2002), and looking at a map of their distributions (eg. Shull et al. 2005) makes it easy to see why; *Euastacus* species are usually limited to a single catchment or mountain top. They are most commonly found in cold, clear mountain streams and rivers, and their preferred habitat may be at risk due to climate change.

The Murray Cray, *Euastacus armatus* (von Martens 1866), has the widest distribution in the genus, being found in both the Murray and Murrumbidgee Rivers and their tributaries (McCarthy 2005; Gilligan et al. 2007). The species is considered to be remarkably invariant morphologically, even across its broad range (Morgan 1986).

Euastacus woiwuru (Morgan 1986) is a small species which occurs on both sides of the Great Dividing Range in Victoria. This species is found in the Dandenong ranges near Melbourne, as well as in central and northern Victoria. Its most closely related species is *Euastacus kershawi* (Smith 1912),

FIG. 1. A, the marbled *Euastacus* and B, *E. armatus*, both found at Schultz Track on the East Buffalo River.

the Gippsland spiny crayfish (Shull *et al.* 2005). Both *E. armatus* and *E. woiwuru* are found in the Buffalo River in northeast Victoria.

In 2002 we visited Dandongadale, on the Buffalo River near the junction of the Rose River, in order to recollect *Euastacus woiwuru* that had first been collected at that locality by P. Horwitz in 1982 (Morgan 1986). When we searched the area this time we could only find *E. armatus*. We continued to search upstream and found an unusual population of crayfish at Schultz Track in 2002 with a marbled carapace (Fig. 1). Further searches of the Buffalo River were interrupted by the bushfires of 2003, which closed some roads for 18 months. When we were able to return to the site, it had been altered by bulldozers and the water was affected by heavy erosion and ash from the fire. We were unable to find any crayfish at that time. In 2006 the fires came again, with heavy fire-fighting taking place near Schultz Track. When the roads opened again in 2007, we were finally able to collect a number of crayfish at Schultz Track and upstream of the site. We were also thus able to determine that the marbled population of *Euastacus* at the Schultz Track site actually occurred at the boundary between *E. armatus* and *E. woiwuru* populations.

The marbled *Euastacus* at Schultz Track have a distinctive colour pattern on the carapace (Fig. 1), and a few other characters that differ from the other spiny crayfish species in the river. We originally misidentified these crayfish as *Euastacus crassus* (Riek 1969) and included the DNA in a large phylogeny of *Euastacus* (specimen number KC2654 in Shull et al. 2005), however, its position on the phylogeny showed clearly that it was not *E. crassus* (Shull et al. 2005). The specimen was then sent to Dr John Short of the Queensland Museum, who said he believed it to be a new species (pers. com.). As such it was listed as an undescribed species in a recent review of the conservation status of Victorian freshwater crayfish (O'Brien 2007).

For measures to be invoked toward the management of crayfish populations, species have to be recognised as threatened and in need of protection (Merrick 1997). The conservation status of the unknown crayfish at Schultz Track could not be clarified until its taxonomic status was resolved. Further collections were interrupted by road closures due to wild fires in 2003 and 2006. We were finally able to collect more individuals in 2007, and discovered that the site where we found the unusual population occurred on the species boundary between *E. armatus* and *E. woiwuru*. In this study we compare the unusual crayfish with other local *Euastacus* species using both morphological and genetic characters.

METHODS

Description of the study site. The Buffalo River flows north from the Barry Mountains and joins the Ovens River near Myrtleford, Victoria.

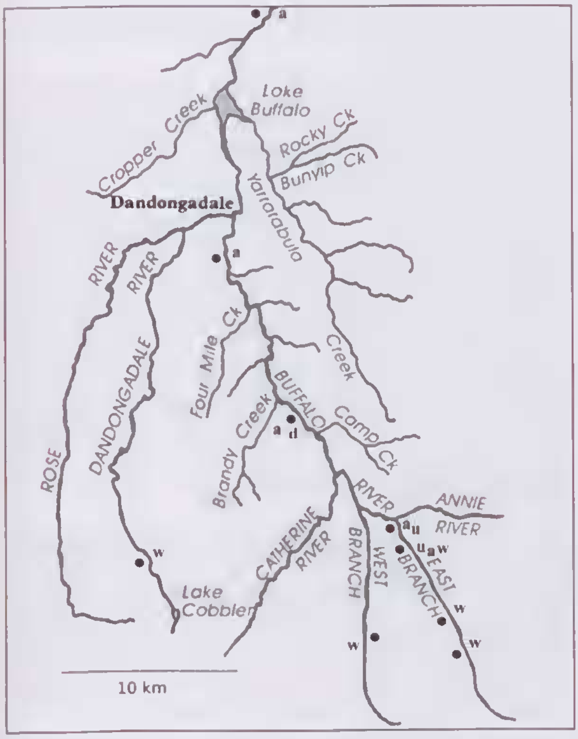

FIG. 2. A map of the Buffalo River showing the locations of Lake Buffalo and Dandongadale. The letters a, u and w indicate sites where *E. armatus*, the unknown (or marbled *Euastacus*) and *E woiwuru* have been collected, respectively. The letter d indicates *Cherax destructor.*

South of Lake Buffalo (a man-made reservoir), the Buffalo River is adjacent to pine plantations and cattle stations as well as State Forest and the Alpine National Park. Schultz Track, where we found the marbled *Euastacus*, is in the East Branch of the upper Buffalo River, about a kilometre above where the East and West Buffalo join (Fig. 2).

Collection of specimens. Specimens were collected using drop nets, bait nets, dip nets and by hand. Concerns for the conservation of this population meant that only two individuals could be retained from each site, so some animals were released after a portion of a leg was removed for DNA analysis. Because they can grow their legs back, this allowed us to get genetic samples without killing the animals. It did, however, limit the number of specimens available for taxonomic work. Some specimens were collected and released live without removing any legs. They were used to record the distribution of crayfish in the river (Fig. 2) but are not part of the material examined.

Material collected but not retained. *Cherax destructor:* Buffalo River, Manna Gum Campsite, VIC, (36°50'S, 146°39'E), 5 Mar. 2002, G. Edney, ♀.

Euastacus armatus. Buffalo River, 1.5 km upstream of Schultz Track, VIC (37°00'S, 146°49'E), 1 May 2007, G. Edney, 2 , ♀.

Taxonomic and morphometric examinations. A total of 45 crayfish were examined for 38 characteristics and 15 measurements that were turned into ratios following to Morgan (1986, 1987 1997). Twenty eight of these were *E. armatus*, seven were *E. woiwuru*, three were *E. crassus*, one was *E. reiki* and six were marbled *Euastacus* from the East Buffalo River. A Categorical Principal Component Analysis (CATPCA) was performed using SPSS version 15 to determine which characters best distinguished species.

Material examined for morphological characters. Currently in the research collection in the Department of Environmental Management and Ecology, but will be deposited in the Museum of Victoria after current ongoing research is completed.

Euastacus armatus. Tallangatta Creek, VIC, (36°17'S, 147°33'E), 20 Apr. 1995, S. Lawler, 5 ♂, ♀; Koetong Creek, VIC, (36°06'S, 147°27'E), 15 May 1995, G. Closs, ♂, 3 ♀♀; Hinces Creek, Burrowa Pines N.P., NSW, (36°05'S, 147°46'E), 26 April 1995, G. Closs & M. Shirley, 5 ♂, 4 ♀; Murray River, Barnawartha VIC, (36°02'S, 146°45'E), 11 July 1995, J. Sloan, ♀; King River, Oxley, VIC, (36°27'S, 146°22'E), 24 June 1995, M. Versteegen, ♂; Tumbarumba Creek, Tumbarumba, NSW, (35°51'S, 148°02'E), 9 July 1995, M. Versteegen, 2 ♂, ♀; Nug Nug, Buffalo River, VIC, (36°40'S, 146°41'E), 6 Dec. 1996, P. Suter, ♀; Ovens River, VIC, (36°02'S, 146°11'E), Sep. 1999, B. Holloway, ♀; Manna Gum Campsite, Buffalo River, VIC, (36°50'S, 146°39'E), 5 Mar. 2002, G. Edney, ♂; Schultz Track, East Buffalo River, VIC, (36°59'S, 146°48'E), 10 Mar. 2002, G. Edney, ♂.

Euastacus woiwuru. Rollason's Falls, Mt. Buffalo NP, VIC, (36°42′S, 146°47′E), 15 Feb. 2000, M. Chapman, ♂; Dobson's Creek, Fern Tree Gully, VIC, (37°52′S, 145°19′E), 23 Mar. 2002, K. Sewell, ♂; West Buffalo River, VIC, (37°02′S, 146°46′E), 23 Nov. 2006, G. Edney, 2 ♂; East Buffalo River, 1.5 km upstream of Schultz Track, VIC, (37°00′S, 146°49′E), 30 Apr. 2007, G. Edney, ♂; East Buffalo River, 4.5 km upstream of Schultz Track, VIC, (37°01′S, 146°49′E), 15 Jul. 2007, G. Edney, ♂; Dandongadale River, near Lake Cobbler, VIC, (37°01′S, 146°37′E), 30 Aug. 2007, G. Edney, ♀.

Euastacus rieki. Tumbarumba Creek, Tumbarumba, NSW, (35°51′S, 148°02′E), 9 July 1995, M. Versteegen, ♂;

Euastacus crassus. Basalt Hill, Falls Creek, VIC, 17 Jan. 2006, D. Heinze, ♂; Native Dog Flat, Buchan River, VIC, (36°90′S, 148°09′E), 26 Feb. 2000, G. Edney, ♂; Tributary of Big River, Dartmouth Dam, VIC, (36°39′S, 147°18′E), 26 Jan. 2007, G. Edney, ♀.

Marbled *Euastacus*. Schultz Track, East Buffalo River, VIC, (36°59′S, 146°48′E), 10 Mar. 2002, G. Edney, QMW 26596, KC2654, ♂; Schultz Track, East Buffalo River, VIC, (36°59′S, 146°48′E), 10 Mar. 2002, G. Edney, ♀; Schultz Track, East Buffalo River, VIC, (36°59′S, 146°48′E), 10 Jul. 2002, G. Edney, ♀; Schultz Track, East Buffalo River, VIC, (36°59′S, 146°48′E), 21 Apr. 2007, G. Edney & S. Lawler, ♀; Schultz Track, East Buffalo River, VIC, (36°59′S, 146°48′E), 22 Apr. 2007, G. Edney & S. Lawler, 2 ♀.

Genetic analysis. The mitochondrial genes COI and 16S were used because they have been used extensively to clarify taxonomy and examine evolutionary processes in freshwater crayfish (Crandall et al. 1995, 1999; Versteegen & Lawler, 1997; Lawler & Crandall 1998; Hughes & Hillyer 2003; Austin et al. 2003; Munasinghe et al. 2003; Shull et al. 2005; Gouws et al. 2006; Ponniah & Hughes 2004, 2006). These gene regions have been used to find cryptic species in other freshwater macroinvertebrates (Chenoweth & Hughes 2003; Baker et al. 2004).

DNA was extracted from tissue (usually gill or a bit of a leg) using guanidium iso-thiocyanate (GIT) buffer and a phenol-chloroform extraction as in Crandall et al. (1995). The DNA was resuspended in 100μl, two microlitres of which was used as the template for a polymerase chain reaction (PCR).

Two different PCR products were amplified from the mitochondrial genome: 720 base pairs of the cytochrome c oxidase subunit I (COI) and 503 base pairs of the 16S rRNA. COI was amplified using the LCO1490 and HCO2198 primers from Folmer et al. 1994. The 16S rRNA fragment was amplified using the 16sL and 1472 primers from Shull et al. 2005. A BioRad PTC-0200 DNA Engine Peltier Thermal Cycler was used to amplify the DNA, with details of the reaction mixtures and temperature profiles available from Street 2007. PCR products were sent to Macrogen Inc. (Seoul, Korea) for single extension DNA sequencing.

Material used for Mitochondrial DNA Amplification. *Euastacus armatus*. Arm1= Manna Gum Campsite, Buffalo River, VIC, (36°50′S, 146°39′E), 5 Mar. 2002, G. Edney, ♂; Arm2 = Ovens River, VIC, (36°02′S, 146°11′E), Sep. 1999, B. Holloway, ♀; Arm3 = Brad Betts Memorial, Yackandandah Creek, VIC, (36°20′S, 146°48′E), 29 Jun. 2007, G. Edney; Arm4 = Buffalo River, VIC, 10 Mar. 2002, G. Edney, QMW26582, KC2653.

Euastacus woiwuru. Woi1 = ♂; East Buffalo River, 1.5 km upstream of Schultz Track, VIC, (37°00′S, 146°49′E), 30 Apr. 2007, G. Edney, ♂; Woi2 = West Buffalo River, VIC, (37°02′S, 146°46′E), 23 Nov. 2006, G. Edney, ♂; Woi3 = East Buffalo River, 4.5 km upstream of Schultz Track, VIC, (37°01′S, 146°49′E), 15 Jul. 2007, G. Edney, ♂; Woi4 = Dobson's Creek, Fern Tree Gully, VIC, (37°52′S, 145°19′E), 23 Mar. 2002, K. Sewell, ♂; Dandongadale River, near Lake Cobbler, VIC, (37°01′S, 146°37′E), 30 Aug. 2007, G. Edney, ♀; Woi5 = West Buffalo River, VIC, (37°02′S, 146°46′E), 23 Nov. 2006, G. Edney, ♂.

Euastacus crassus. Cra1 = Native Dog Flat, Buchan River, VIC, (36°90′S, 148°09′E), 26 Feb. 2000, G. Edney, ♂; Cra2 = Native Dog Flat, Buchan River, VIC, (36°90′S, 148°09′E), 19 Mar. 2002, G. Edney, KC2649, ♂; Cra3 = Native Dog Flat, Buchan River, VIC, (36°90′S, 148°09′E), 20 Mar. 2002, G. Edney, KC2720. *Euastacus yarraensis*. Yar 1 = Love Creek, VIC, (38°48′S, 143°58′E), 1 Jan. 2004, K. Sewell & G. Edney, KC2831; Yar 2 = Cockatoo, VIC (37°94′S, 145°49′E) 21 Mar. 2002, KC2651.

Marbled *Euastacus*. Unk1 = Schultz Track, East Buffalo River, VIC, 10 Jul. 2002, G. Edney, ♀; Unk2 = Schultz Track, East Buffalo River, VIC, 10 Mar.

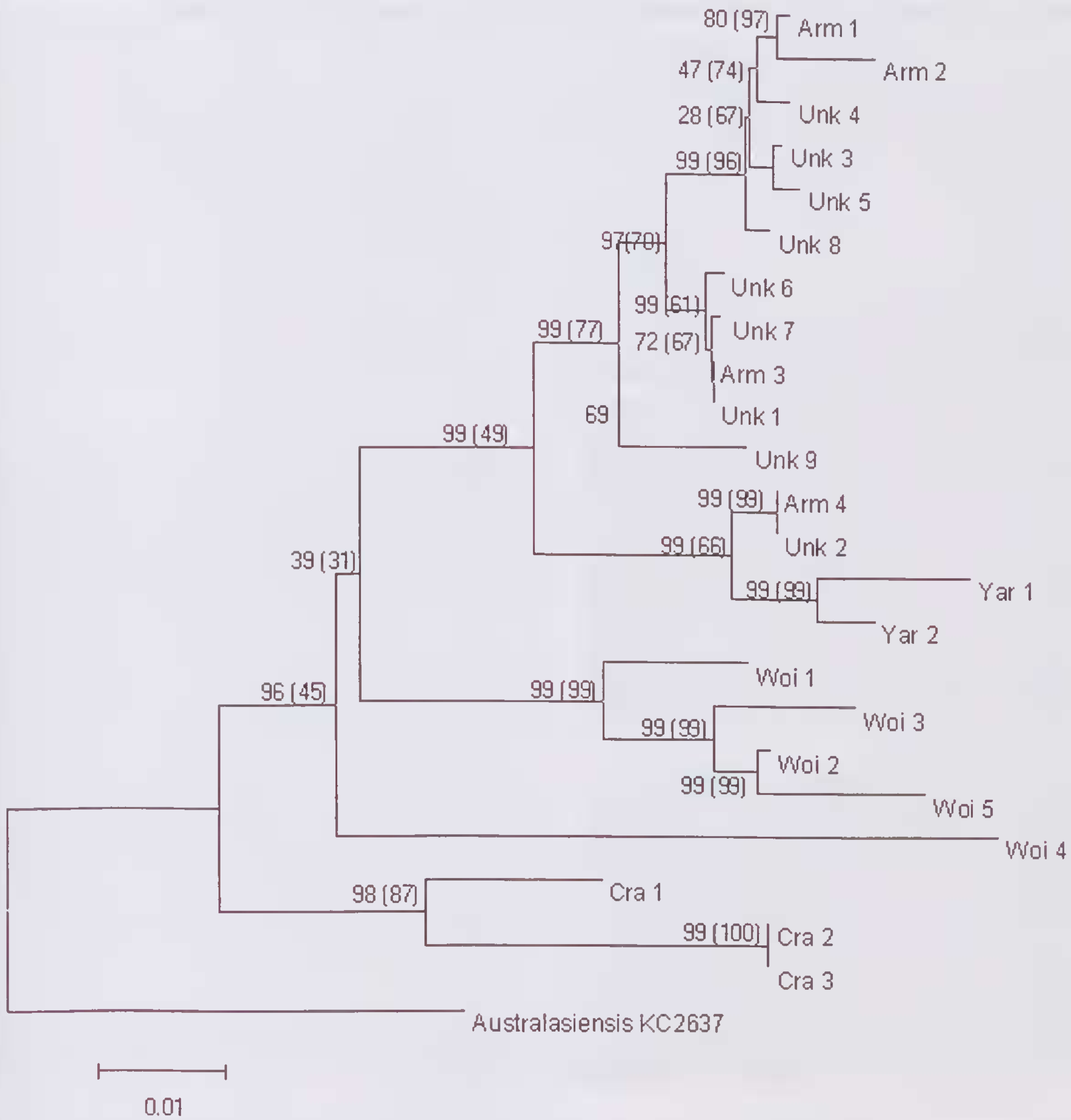

FIG. 3. Neighbour-joining consensus tree using 16S and COI mitochondrial DNA sequences. Interior branch test probabilities are shown on nodes with bootstrap values shown in parentheses, both using 100,000 replications. Arm = *E. armatus*, Unk = Marbled *Euastacus*, Woi = *E. woiwuru*, Yar = *E. yarraensis*, Cra = *E. crassus* and the outgroup is *Euastacus australasiensis*. For information on collection sites see methods section.

TABLE 1. Some morphological characters of the *Euastacus* of the Buffalo River, Victoria. The characters for *E. armatus* and *E. woiwuru* agree with Morgan (1986), who examined many more individuals.

	Marbled *Euastacus*	*E. armatus*	*E. woiwuru*
Number examined	6	28	7
TAP	4 – 5	5 – 7	7 – 9
Urocardiac Ridge	8	9 – 10	9 – 11
Marginal mesal dactylar spines	0 – 1	0 – 3	0 – 1
Dorsal mesal dactylar spines	0 – 5	0 – 3	2 – 5
Mesal carpal spines	2	2	3
Male cuticle partition	no	no	yes
Telsonic spines	yes	yes	no

2002, G. Edney, QMW 26596, KC2654, ♂; Unk3 = Schultz Track, East Buffalo River, VIC, 22 Apr. 2007, G. Edney & S. Lawler, ♀; Unk4 = East Buffalo River, 1.5 km upstream of Schultz Track, VIC, (37°00′S, 146°49′E), 30 Apr. 2007, G. Edney & D. Street (leg only); Unk5 = East Buffalo River, 1.5 km upstream of Schultz Track, VIC, 1 May 2007, G. Edney & D. Street (leg only); Unk6 = East Buffalo River, Schultz Track, VIC, 22 Apr. 2007, G. Edney & S. Lawler; Unk7 = East Buffalo River, Schultz Track, VIC, 22 Apr. 2007, G. Edney & D. Street (leg only); Unk8 = Schultz Track, East Buffalo River, VIC, (36°59′S, 146°48′E), 21 Apr. 2007, G. Edney & S. Lawler, ♀; Unk9 = Schultz Track, East Buffalo River, VIC, 3 July 2007, G. Edney & D. Street, (leg only).

Phylogeny construction. DNA sequences were aligned using Cluster W in the computer program MEGA Version 4.0 (Kumar et al., 2004; Tamura et al., 2007). Only specimens that were successfully sequenced for both mitochondrial gene regions were used for phylogeny construction. *Euastacus australasiensis* (KC2637) was used as an outgroup, and other sequences were included for comparison, including the marbled *Euastacus* KC2654, *E. armatus* KC2653, *E. crassus* KC2720 and *E. yarraensis* KC281 and KC 2651 (Shull et al. 2005).

A consensus tree for the two gene regions was constructed using the neighbour joining method in MEGA. Two different probabilities, the bootstrap and interior branch test, were measured for each node on the phylogeny (Fig. 3).

RESULTS

Distribution. We found a total of 10 crayfish in the Buffalo River that, while clearly belonging to the genus *Euastacus*, did not resemble any known species. Three of these 'marbled' *Euastacus* were collected in 2002 and seven in 2007. Four of the animals caught in 2007 were released on site after removing a portion of a leg for genetic analysis. All of these animals were found in a stretch of river only a few kilometres long in the East Buffalo River (Fig. 2).

Euastacus armatus are widely distributed in the Buffalo River below the lake, and in the Ovens River into which the Buffalo River empties. They were also found near Dandongadale (the locality), at Schultz Track, and 1.5 km upstream of Schultz Track, where they occurred in sympatry with *E. woiwuru*.

Euastacus woiwuru were found on top of Mount Buffalo, where they had not been previously recorded, but were not found near Dandongadale where they had been recorded in 1982 (Morgan 1986). They were also found at Schultz Track and 1.5 km, 4.5 km, and 6.8 km upstream from Schultz Track in the East Buffalo River. They were also found in the West Buffalo River and in the upper Dandongadale River, so their distribution is in the headwaters of the catchment (See Fig. 2). Both *E. armatus* and *E. woiwuru* are found in sympatry with the marbled *Euastacus* 1.5 km upstream of Schultz Track.

The common yabby, *Cherax destructor*, was found in the Buffalo River, 6 km south of Dandongadale (Fig. 2), and *Engaeus* burrows are common throughout the catchment. We have not dug up any burrows or actively sampled the burrowing crayfish, but the species is probably *Engaeus cymus* (Clark 1936) (Horwitz 1990b).

During the taxonomic examinations of old collections, we found another case of *Euastacus armatus* in sympatry with another species of *Euastacus*. A collection from a single site (Tumbarumba Creek, NSW in 1995) was found to contain both *E. armatus* and *Euastacus reiki* (Morgan 1997). These species both have white claws and similar spination, highlighting the degree to which different species can appear similar in this genus. One of the characters used to distinguish *E. reiki* from *E. armatus* is the TAP count.

Morphological examinations. A total of 45 *Euastacus* specimens were scored for 38 characteristics and 15 measurements that were turned into ratios according to Morgan (1986, 1987, 1997). Our ratios fell well within the range of Morgan's data for all the species examined, and hierarchical cluster analyses in SPSS grouped the animals into species clusters (Street 2007). The CATPCA test in SPSS produced a list of morphological characters that were most useful in differentiating these species of *Euastacus*. A shorter list emphasising the morphological differences in the Schultz Track population is shown in Table 1.

The characters used to distinguish the three *Euastacus* species were the male cuticle partition, telsonic spines, mesal carpal spines and the TAP (Morgan, 1986, 1997). The number of teeth anterior to the posterior margin of the zygocardiac ossicle ear, or TAP, is a morphological character that was described by Francois (1962), is considered to be a useful character for crayfish taxonomy (Growns & Richardson 1990) and has been used in the description of *Euastacus* species (Morgan 1986; 1988; 1997). The TAP of *E. armatus* is between 5 and 7, while the range of TAP in *E. woiwuru* is between 7 and 9 (Morgan 1986).

The Schultz Track population, or the marbled *Euastacus*, had TAPs at or below the range of *E. armatus*, with at least half of them having five teeth in one ear and four in the other. These assymetrical individuals were given a TAP score of 4.5, while somel individuals had four teeth in both ears, which is outside of the published range of *E. armatus* (Table 1).

All of the six marbled specimens examined for morphology were small, with occipital carapace lengths (OCLs) less than 44 (28, 28, 28, 29, 37 43). The larger ones had white on the tips of their claws. It is possible that they develop white claws as they grow larger. *E. armatus* also change from green or brown claws to white as they grow, but the size where this change occurs seems to vary between populations (pers. obs., SL).

Genetic analysis and phylogeny. Phylogenies constructed for the two mitochondrial DNA regions (16S and COI) produced the same topology. The consensus tree is shown in Fig. 3. The phylogeny shows all the marbled *Euastacus* in the same clade with *E. armatus*. According to the mitochondrial DNA phylogeny, the unusual *Euastacus* is not a separate species from *E. armatus*, however, DNA analysis using nuclear genes (Ji et al. 2003) may yet show that this population is different.

DISCUSSION

An unusual population of crayfish of the genus *Euastacus* was found in a very short stretch of river. This population is designated the marbled *Euastacus* in this paper, and its taxonomic status remains unclear. The location of this population between two different species (Fig. 2) suggests that it could be a hybrid.

Genetic sequences mitochondrial DNA genes 16S and COI were used to test the hypothesis of a hybrid zone. These genes were chosen because they have been used for many studies of speciation and population structure of Australian freshwater decapods (Crandall et al., 1995; Hughes and Hillyer, 2003; Chenoweth & Hughes, 2003; Shull et al., 2005; Schultz et al., 2007).

The use of the mitochondrial gene cytochrome oxidase (COI) to identify species is also known as barcoding (Mitchell 2008). Although it has been used to identify cryptic species in Crustacea (Witt et al. 2006) and in insect groups (Rubinoff & Sperling 2004; Hebert et al. 2004; Smith et al. 2006), there are also instances in which COI has been unable to distinguish between species (Meier et al. 2006; Hickerson et al. 2006; Whitworth et al. 2007). Nevertheless, some authors claim it is effective for measuring hybridisation events, defining hybrid zones and discovering cryptic species (Rubinoff & Holland 2005).

Failures to resolve species boundaries using mitochondrial genes are more likely when the species have recently diverged, or in cases of inter-specific hybridisation (Shaw 2002). Nelson et al. (2007) were able to identify nine species of blowflies using DNA barcoding, but misidentified the one hybrid specimen using this techinique.

Our data clearly separated known species of the genus *Euastacus*, with *E. armatus* and *E. woiwuru* forming well defined monophyletic groups (Fig. 3). The marbled *Euastacus* fell within the *E. armatus* clade using consensus sequences of the mitochondrial genes COI and 16S. This pattern is suggestive rather than conclusive, however, because mitochondrial DNA is inherited directly from the mother without undergoing recombination. If the mothers of the hybrids consistently belong to the species *E. armatus*, this pattern would still occur. Behavioural constraints could result in a bias during inter-specific matings, particularly if the animals involved have a consistent size difference. For example, we know that small males can mate with larger females, and the species *E. armatus* usually grow larger than *E. woiwuru* (Morgan 1986).

Some authors say that *E. armatus* is not found in sympatry with other members of its genus (Gilligan et al. 2007). This perception may be due to the lack of sampling in areas of potential overlap, because we have found *E. armatus* and *E. woiwuru* at the same site in the Buffalo River, and we also identified at least one site in New South Wales where *E. armatus* is found in sympatry with *E. rieki*. We did not recognise the sympatry at the time of collection (in 1995) because *E. rieki* had not yet been described (Morgan 1997). *Euastacus reiki* and *E. armatus* are so similar morphologically that we have to dissect the gastric mill to tell them apart, which makes them very hard to distinguish in the field.

Almost 30 years ago, *Euastacus woiwuru* were found near Dandongadale, but only Murray Crays (*E. armatus*) are found there now. *Euastacus woiwuru* are now found about 20 kms south of Dandongadale in the East and West branches of the Buffalo River. The apparent movement of the Murray Cray upstream could be explained by the alteration of habitats by human disturbance or by the warming of the river due to climate change or many years of drought. Either way, the change in distribution may indicate that the species boundary between *E. armatus* and *E. woiwuru* has been moving for decades. The fact that an unusual morphological variant is found at this boundary is highly suggestive of a hybrid zone. However, if they are hybrids, they do not appear to form a self sustaining breeding population, because both parent species are present at the localities where the marbled form is found.

Marbled *Euastacus* are morphologically distinct in their colouration and the fact that some of their TAPs (the number of teeth anterior to the posterior margin of the zygocardiac ossicle ear) are below the published range of *E. armatus* and *E. woiwuru*. Interestingly, Morgan states that the TAPs of *E. woiwuru* are generally lower in Murray River tributaries like the Buffalo River, and that a specimen collected at Dandongadale was unusual (Morgan 1986, p. 49). Perhaps this location, which is at the edge of the distribution of both species, contains some unique morphological variants.

The marbled *Euastacus* were all very small, with OCLs (occipital carapace length) below 44 mm. However, since the larger of the marbled *Euastacus* had white tips on their claws, it is possible that the marbled crays are a unique juvenile variant, and that these animals grow into adult Murray Crays with white claws and a uniformly coloured carapace. Normally, juvenile Murray Crays do not have the white claws that are a distinctive feature of the adults, but neither do they have a marbled pattern. The substrate at this site did not obviously differ from other stretches of the river where *E. armatus* juveniles do not show this marbled pattern, but it is possible that there is a yet to be identified environmental factor affecting their colour. Because our permit did not allow us to retain and raise these animals, we were unable to confirm the adult colouration of the marbled specimens.

Many threatening processes are controllable, but for measures to be invoked toward the management of crayfish populations, species have to be recognised as threatened and in need of protection (Merrick 1997). The sedentary nature of crayfish and limited gene flow between catchments (Fetzner & Crandall 2001; Gouin et al. 2006) makes them susceptible to over-fishing and habitat alteration (Merrick 1997; O'Brien 2007). The first step toward listing a population is to determine if it is different enough to be considered a conservation unit (Fraser & Bernatchez 2001).

Unique fauna are often found in unregulated headwater streams (Baker et al. 2004), and this study provides an example of an unusual population of spiny crayfish. The conservation status of this population needs to be clarified. The location is subject to several threatening processes. Cows are allowed to roam freely in and out of the river, and fires and fire control measures combined to significantly disturb the site in 2003 and 2006. Given the fire history of the area, we can expect reduced water flows in the catchment for decades.

Speciation is a slow process in this genus (Ponniah & Hughes 2006). If we have found an unusual population of *E. armatus*, it could still be different enough to be deserving of conservation. On the other hand, finding this crayfish at the boundary of two *Euastacus* species means that the possibility of hybridisation must be taken seriously, particularly since introgression is a possibility (Ballard & Whitlock 2004; Funk & Omland 2003; Shaw 2002).

We are planning to sequence nuclear genes using ITS primers (Ji et al. 2003) and will continue to make field observations. Investigation of freshwater crayfish species boundaries in the Buffalo River may enlighten us about the ecology and evolution of Australian spiny freshwater crayfish.

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